Microbial spoilage and fish and shellfish diseases cause significant economic loss to the industry and to marine aquaculture. Outbreaks of seafood-borne illness regularly occur in the US. In this project, Vibrio spp. and total bacteria were selected as target pathogens that cause human and fish diseases. The purpose of this project is to develop a rapid Multiplex Rti-PCR assay for the simultaneous detection and quantification of three representative Vibrio spp. and a number of total bacteria. The assay developed in this project could change the way pathogens are detected and provide rapid and cost-effective microbiological methods for the routine monitoring and risk assessment of seafood and marine water quality.

Development of a multiplex real-time PCR assay for rapid quantification of Vibrio spp. and total bacteria in seafood and seawater for multipurpose assessment

Who cares and why?

The US is the third largest global consumer of fishery products. Annually, bacterial spoilage of fishery products results in the loss of one-quarter to one-third of fishery and agricultural products followed by billions of dollars in direct economic losses. Furthermore, pathogenic bacteria in spoiled fish can be a cause of foodborne illness, and represents a risk to public health. Especially, the incidence of *Vibrio* infections due to seafood consumptions has increased by 43% between 2006 and 2012 in the US.

Vibrio parahaemolyticus and *V. vulnificus* are the leading cause of illness from seafood; it has the highest mortality rate, greater than 50% for primary septicemia, and has led to approximately 40 deaths from seafood in the US annually. Also, *Vibrio anguillarum* causes vibriosis with lethal hemorrhagic septicemia in fish and shellfish that result in substantial economic losses in aquaculture farming worldwide.

Although *Vibrionaceae* have generally been detected using traditional cultivation methods, this method does not indicate specific *Vibrio* species. In terms of molecular assay, conventional PCR assays cannot make quantitative measurements but a current real-time PCR method is quantitative, more rapid, and about 100 times more sensitive than the conventional method.

The other concern of food safety is high bacterial populations in and on aquaculture products affect their quality, shelf-life, and suggest the possibility of increased numbers of potential human pathogens; the sushi industry has been growing by 1.6% annually through 2008-2013 and current numbers of sushi restaurants total 4,135 in the US.

Therefore, development of a rapid method for the detection and quantification of three vibrio pathogens (*V. parahaemolyticus, V. vulnificus, and V. anguillarum*) and total bacteria is crucial in aquaculture for the detection of infected fish and to monitor seafood quality in marine environments.

What has the project done so far?

This project has a methodological innovation. This method is able to detect and quantify three specific and one non-specific bacterial spp. simultaneously. A four-target multiplex real-time PCR assay was developed using the Taqman system. The novel primers and probes were designed from species-specific virulence genes for *V. parahaemolyticus, V. anguillarum,* and *V. vulnificus* as well as detection of a universal target gene for total bacteria. This approach is the first attempt for the assessment of fish and marine water safety and quality.

We optimized the multiplex real-time PCR conditions and applied it to detect the total number of bacteria and Vibrio spp. from the aquacultured and wild caught fish as well as water samples. Fish fillets were obtained from the aquaculture facility at Delaware State University (DSU) and local retail sources in Dover, Delaware, USA. The fillets used in this study were: sea bass (*Centropristis striata*), cod fish (*Gadus morhua*), flounder (*Paralichthys*), haddock (*Melanogrammus aeglefinus*), hybrid striped bass (*Morone Chrysops x Morone saxatilis*), mummichog (*Fundulus heteroclitus*), and tilapia (*Oreochromis mossambicus*). Seawater samples

were collected from different locations and on different days. Estuarine water samples were collected from the Indian River inlet in Delaware. Three Delaware Bay seawater samples were collected from Bowers beach, Delaware as well as regular seawater samples were collected from the aquaculture facility at DSU.

The total bacterial populations in fish and seawater quantified by a culture method and multiplex real-time PCR assay were similar and indicate that both methods have a close correlation. Therefore, the multiplex real-time PCR assay would be very practical to enumerate total bacteria in fish and seawater. The total numbers of the 3 Vibrio spp. by multiplex real-time PCR assay was consistently higher than those of traditional plate counts. Selective media is usually used for the traditional isolation of *V. cholerae, V. parahaemolyticus*, and other *Vibrio* spp. but has some limitations. Uncultivable and injured *Vibrio* spp. in environments will not grow on this media because of selective agents. From the results obtained in this study, the quantification of Vibrio spp. in fish and seawater by this multiplex real-time PCR assay appears to be more accurate than culture methods.

To the best of our knowledge, our study describes the first multiplex real-time PCR assay for the simultaneous detection of these three species. Furthermore, this assay quantified total bacteria in the seafood and seawater samples at the same time. To prevent pathogenic *Vibrio* infection to humans and fish, their presence in seafood and seawater should be accurately monitored and total bacterial counts could be used as an indicator for measuring the quality of fishery products. This multiplex real-time PCR assay will facilitate the rapid surveillance of fishe and seawater for *Vibrio* spp. and total bacteria as well as could be applicable as a diagnostic method in seafoodborne outbreak situations.

Impact Statement

The real-time PCR using multi-probes was developed for the simultaneous detection and quantification of three representative *Vibrio* spp. and a number of total bacteria in seafood and water.

The real-time PCR was applied to various fish and water samples derived from Delaware Bay, estuary, and aquaculture facility and showed a close correlation to the traditional culture method.

This multipurpose real-time PCR assay could provide rapid and cost-effective microbiological analysis for the routine monitoring and risk assessment of seafood and marine water quality.

What research is needed?

Aquaculture trials will be required for further study, which includes artificial inoculation of *Vibrio* spp. into aquaculture seawater and the inducing of infections in fish cultured in the aquaculture system. In this trial, we will assess the total bacteria and *Vibrio* spp. in fish and their surrounding aquaculture seawater using the multiplex real-time PCR assay and evaluate their correlation between both subjects in the ecosystem.

Want to know more?

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Strategic Priority: Health and nutrition
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